The association between vesicle-associated membrane protein - 8 A/G gene polymorphism and the risk of acute myocardial infarction

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Abstract

Introduction: Until now, only few studies have reported the correlation between vesicle-associated membrane protein-8 (VAMP-8) A/G gene polymorphism and acute myocardial infarction. Whereas, theoretically, VAMP-8 plays a pivotal role in the pathogenesis of acute myocardial infarction through platelet activation, secretion, and aggregation. Objective: To investigate the association between VAMP-8 A/G gene polymorphism and the risk of acute myocardial infarction. Methods: A cross-sectional study was carried out at Saiful Anwar General Hospital during June 2013 - May 2014. A Mae II enzyme with restriction fragment length polymorphism method was used to genotype VAMP-8 A/G gene polymorphisms in acute myocardial infarction and control groups. A multiple logistic regression test was used to analyze the association between VAMP-8 A/G gene polymorphism and the risk of acute myocardial infarction. Results: A total of 35 controls and 97 acute myocardial infarction patients from our Hospital during the period were enrolled for our study. Our results found that VAMP-8 A/G gene polymorphism was not associated with the risk of acute myocardial infarction. Moreover, we also failed to confer the association between VAMP-8 A/G gene polymorphism and both smoking and hypertension among patients with acute myocardial infarction. Furthermore, in the setting of premature acute myocardial infarction, the correlation also failed to confirm. Conclusion: In our population, there is no association between VAMP-8 A/G gene polymorphism and the risk of acute myocardial infarction.

Key words: Gene Polymorphism. Acute myocardial infarction. Membrane Proteins.

Resumen

Introducción: Hasta la fecha, solo unos pocos estudios han reportado la correlación entre el polimorfismo A/G del gen de la proteína de membrana asociada a vesículas-8 (VAMP-8, por sus siglas en inglés) y el infarto agudo de miocardio. Si bien, en teoría, VAMP-8 juega un papel fundamental en la patogénesis del infarto agudo de miocardio a través de la activación, secreción y agregación plaquetaria. Objetivo: Investigar la relación entre el polimorfismo A/G del gen VAMP-8 y el riesgo de infarto agudo de miocardio.
Introduction

The three main pathological stages in coronary artery disease (CAD) are the process in pre-intima, in-intima, and post-intima. Of these, the pathological mechanism in post-intima—defined as platelet activation, secretion, and aggregation—is the most responsible to cause clinically acute myocardial infarction. This process involves several proteins including von Willebrand factor (vWF), thromboxane A2, collagen, fibrinogen, and vesicle-associated membrane protein-8 (VAMP-8). Of these, studies concerning the correlation between VAMP-8 and acute myocardial infarction had a limited number. Whereas, elevated levels of VAMP-8 had been shown to be correlated with platelet hyperreactivity and overexpression, platelet granule secretion, and thrombus formation. Moreover, by evaluating the levels of VAMP-8 messenger ribonucleic acid (mRNA), a study found that VAMP-8 mRNA levels were higher in hyperreactive platelets.

Recently, several single-nucleotide polymorphisms (SNPs) of VAMP-8 gene have been reported, such as rs1010, rs1058588, rs1009, rs13421434, rs1348818, rs3731828, rs7579147, rs3770098, and rs6757263. Therefore, this raises an assumption that rs1010 (VAMP-8 A/G) is merged with other SNPs, for example rs16988, rs1058601, rs3199256, rs17617682, rs56477175, and rs59206761. Interestingly, in the SNP database (https://www.ncbi.nlm.nih.gov/snp/?term=vamp8), it was showed that rs1010 (VAMP-8 A/G) is merged with other SNPs, for example rs16988, rs1058601, rs3199256, rs17617682, rs56477175, and rs59206761. Therefore, this raises an assumption that rs1010 (VAMP-8 A/G), located at the 3’ untranslated region, may have a pivotal role for VAMP-8 formation and may correlate with VAMP-8 levels in the circulation. Some studies had reported the association between VAMP-8 A/G gene polymorphism and the risk of CAD in some countries. However, in our country, the study in this context has never been conducted.

Our present study aimed to investigate the correlation between VAMP-8 A/G gene polymorphism and the risk of acute myocardial infarction in our Hospital (Saiful Anwar General Hospital). This is the first report concerning the association between VAMP-8 A/G gene polymorphism and the risk of acute myocardial infarction in Indonesia and Southeast Asian countries.

Method

Study designs and patients

To assess the association between VAMP-8 A/G gene polymorphism and the risk of acute myocardial infarction, we performed a cross-sectional study at Saiful Anwar General Hospital, Malang, Indonesia from June 2013 to May 2014. The target population was all men subjects with acute myocardial infarction treated in our Hospital during the period. Men subjects aged ≥ 30 years with acute myocardial infarction were included in the study. Several parameters including clinical conditions, electrocardiography, myocardial enzyme, and angiography were used to confirm acute myocardial infarction diagnosis. While, patients with diabetes mellitus (DM), infection, impaired renal function, neoplasm, and subjects who disagree to give blood for the study were excluded from the study. Controls were healthy age-and-sex-matched subjects in our population (population-based). The blood samples from the peripheral vein were collected (10 ml), then were put in EDTA-coated tubes and kept cold at -14°C. All patients had signed the informed consent before participating in the study. This study was approved by Ethical Committee of Universitas Brawijaya, Malang, Indonesia and carried out in accordance with Declaration of Helsinki for humans experiments.
Briefly, the genotype frequency was determined for all the cases and controls. The protocols were adapted from previous studies with some modifications. A set of primers (sense: 5’- GGG GGC TCC AAC TTT CTT CTC C and antisense 5’- CTT TGC CAC TGG TGC CTT CTC TTA) was designed to identify VAMP-8 A/G gene polymorphism. We amplified the DNA (Perkin Elmer 2400, Boston, USA) for 35 cycles and each cycle consisted of pre-denaturation at 95 °C for five minutes, denaturation at 98 °C for 20 seconds, annealing at 61 °C for 15 seconds, elongation at 72 °C for 45 seconds, and post-elongation at 72 °C for five minutes.

To determine VAMP-8 A/G gene polymorphism, Mae II enzyme with Restriction Fragment Length Polymorphism (RLFP) method was used. A 10 µL of total reaction volume contained 5.65 µl ddH2O, 1 µl Buffer Y, 0.35 µl Mae II enzyme, and 3 µl DNA PCR product. The mixture was then incubated at 65 °C for three hours. For G variant, the products of 328 bp and 166 bp were digested. While, for the A allele, the final product of 494 bp remained undigested. RFLP products were electrophoresed using a 2% agarose gel (Hoeffer, Holliston, USA), stained with ethidium bromide, and analyzed digitally using Gel Doc™ EZ System (Gel Doc, California, USA).

The association between VAMP-8 A/G gene polymorphisms and the risk of acute myocardial infarction was analyzed using multiple logistic regression. Statistically significant was considered if the P-value was less than 0.05. The Statistical Package of Social Sciences 17.0 software (SPSS Inc., Chicago, IL) was used to analyze the data.

During the period, a total of 144 patients with acute myocardial infarction was treated in our Hospital. Of those, two patients were excluded because of aged under 30 years; 18 patients were excluded because of DM; five patients were excluded because of having infection; 11 patients were excluded because they had renal disease; three patients were excluded because of neoplasm, and we also excluded eight patients because they disagreed for the study. Finally, a total of 97 patients and 35 controls were included in the study.

The frequencies of VAMP-8 A/G gene polymorphism in acute myocardial infarction and control groups are presented in Table 1.
described in Table 2. RLFP for VAMP-8 A/G gene polymorphism is described in Figure 2. For acute myocardial infarction group, the frequency of GG, GA, and AA genotypes were 23, 44, and 30; respectively. While, for control group, the VAMP-8 A/G genotypes frequency were 5, 15, and 15 for GG, GA, and AA; respectively. Our genotype frequencies conformed with Hardy-Weinberg equilibrium both in case ($X^2$ for HWE = 0.75) and control ($X^2$ for HWE = 0.16). Our results showed that VAMP-8 A/G gene polymorphism was not associated with the risk of acute myocardial infarction.

Furthermore, for subgroup analysis, we also analyzed several factors including smoking, hypertension, and early acute myocardial infarction in our study. The
The frequency of GG, GA, and AA was 22, 33, and 24, respectively, for the smoking group, and 1, 11, and 6, respectively, for the non-smoking group (Table 3). For acute myocardial infarction patients with hypertension (Table 4), the frequency of VAMP-8 A/G gene polymorphism was 10, 22, and 11 for GG, GA, and AA, respectively. While, for acute myocardial infarction patients without hypertension, the frequency was 13, 22, and 19 for GG, GA, and AA, respectively. For acute myocardial infarction patients aged under 55 years, the frequency of GG, GA, and AA was 13, 15, and 10, respectively. While, for acute myocardial infarction patients aged more than or equal to 55 years, the frequency was 10, 29, 20, respectively (Table 5). Our analysis found that no correlation was observed between VAMP-8 A/G gene polymorphism and those several factors.

**Discussion**

To date, genetic studies in the context of acute myocardial infarction have been widely reported. Of these, studies concerning VAMP-8 A/G gene polymorphism have a limited number. Nevertheless, theoretically, VAMP-8 is highly associated to acute myocardial infarction pathogenesis through platelet activation, secretion, and aggregation. In our country, until now, no study reported VAMP-8 A/G gene polymorphism. Our present study reported VAMP-8 A/G gene polymorphism between acute myocardial infarction and control groups. Our results found that VAMP-8 A/G gene polymorphism was not associated with the risk of acute myocardial infarction. Theoretically, VAMP-8 has a crucial role in platelet activation through stimulating platelet
exocytosis and platelet-dense granule secretion\(^\text{10}\). This may lead to the fusion between granule membrane and platelet plasma membrane, and also fusion among granules\(^\text{26}\). This fusion is facilitated by membrane protein, called v-soluble N-ethyl maleimide sensitive factor attachment protein receptor (v-SNARE) (SNARE in granule) and t-SNARE (SNARE in targeted membrane). v-SNARE and t-SNARE may form two layers of hetero-meric complex leading to membrane fusion and release granule content. As the results, platelet may be activated\(^\text{27}\). It has been widely known that platelet activation plays a pivotal role in the development of acute myocardial infarction\(^\text{25}\). Therefore, theoretically, VAMP-8 A/G polymorphism may have the association with the risk of acute myocardial infarction. However, our study failed to support this theory. Further investigations with comprehensive methods are required to elucidate this correlation. Totally, based on searching in Pubmed and EMBASE, there were nine studies evaluating the correlation between VAMP-8 gene polymorphism and the risk of CAD. Our results were consistent with Luke et al.\(^\text{15}\), van der Net et al.\(^\text{16}\) and Akao et al.\(^\text{17}\) but contrast with Liu et al.\(^\text{18}\), Bare et al.\(^\text{19}\), Shiffman et al.\(^\text{20}\), Shiffman et al.\(^\text{21}\), Duan et al.\(^\text{22}\), and Ke-jun et al.\(^\text{23}\). Although some studies had proven the correlation between VAMP-8 gene polymorphism and the risk of CAD. Our results were consistent with Luke et al.\(^\text{15}\), van der Net et al.\(^\text{16}\) and Akao et al.\(^\text{17}\) but contrast with Liu et al.\(^\text{18}\), Bare et al.\(^\text{19}\), Shiffman et al.\(^\text{20}\), Shiffman et al.\(^\text{21}\), Duan et al.\(^\text{22}\), and Ke-jun et al.\(^\text{23}\). Although some studies had proven the correlation between VAMP-8 gene polymorphism and the risk of CAD. However, it was not clear whether A or G allele had the impact on increasing the risk of CAD. Two studies had been conducted in the Chinese Han population, but showed different results. A study\(^\text{22}\) showed that A allele was correlated with an increased risk of CAD. While, another study\(^\text{15}\), although used inhomogeneous sample, found G allele. Moreover, although the association between VAMP-8 gene polymorphism and the risk of CAD was also reported by Bare et al.\(^\text{19}\), however, they had no control. They compared their results with control of other studies. Therefore, some factors including different population and region may affect the correlation, and study bias remained to be considered. Furthermore, some studies\(^\text{16,23}\) included female with or without post-menopause subjects. As well known that menopausal factors have proven to contribute in increasing the risk of CAD\(^\text{28}\), and therefore this factor may cause study bias. In addition, some studies data were not presented in Hardy-Weinberg equilibrium and fulltexts were not available\(^\text{20,23}\). Therefore, the data could not be analyzed further. In our present study, we designed our study with eliminating these limitations factors. Therefore, we expected that our results might provide the better outcome. Moreover, due to these reports remain conflicting, we calculated odds ratio and 95 confidence interval (OR 95%CI) of five studies, including our results, to conclude the association. The pooled calculations found that no correlation was observed between VAMP-8 gene polymorphism and the risk of CAD (Fig. 3A). However, our calculation is not the final. In the near future, we expect that there will be the studies evaluating this topic with specific design especially meta-analysis.

For sub-group analysis, we also evaluated the correlation between VAMP-8 gene polymorphism and several factors including smoking, age, and hypertension among acute myocardial infarction patients. Theoretically, these factors have pivotal role in VAMP-8-related to acute myocardial infarction. During this time, no study reports the direct correlation between VAMP-8 and
smoking. However, the possible mechanism has been proposed. In smoker patients, FXIII is up-regulated. The adhesion of activated platelet to FXIII, mediated by GpIIb IIIa receptors, is one of platelet aggregation pathways. While, platelet secretion and activation are regulated by VAMP-8. This process is the beginning of thrombus formation in CAD patients. However, our results showed otherwise, no correlation was observed between VAMP-8 A/G gene polymorphism and smoking among acute myocardial infarction patients. Moreover, for the correlation between VAMP-8 and hypertension, it has been reported that, by mediating normal granule maturation, VAMP-8 inhibits renin release. Renin-angiotensin-aldosterone-system is the pathway responsible for hypertension. This means that elevated level of VAMP-8 has protective role against hypertension. In this context, we also failed to show the correlation. In the previous studies, these factors were not involved in the analysis. Therefore, the correlation between VAMP-8 gene polymorphism and both smoking and hypertension among acute myocardial infarction patients was unknown. For this reason, we could not compare our outcome to the previous studies, either systematically or narratively. Moreover, data in the literature was not enough to elaborate the possible reason.

Furthermore, for the association between age and VAMP-8 A/G gene polymorphism, several studies had reported the correlation between early acute myocardial infarction and VAMP-8 A/G gene polymorphism. This correlation is assumed that VAMP-8 may cause the reduction of stem-loop structure stability. As the results, this leads to plaque destabilization which triggers to early acute myocardial infarction. Although they showed that VAMP-8 A/G polymorphism was associated with early acute myocardial infarction, however, whether the A or G allele correlating to the risk of early acute myocardial infarction is still inconclusive. Shiffman et al. showed that G allele was found to be correlated with the risk of early acute myocardial infarction, while Goracy et al. found A allele. Moreover, the definition of early acute myocardial infarction in the previous studies was unclear, ranging from less than 45 to 60 years old. For this reason, we defined early acute myocardial infarction as subjects with age less than 55 years old, and our results found that no association between early acute myocardial infarction and VAMP-8 A/G polymorphism. Our results were contrast with those previous studies. Due to this difference, we combined our data with Goracy et al., and we also found that no correlation was observed between early acute myocardial infarction and VAMP-8 A/G polymorphism (Fig. 3B). However, early acute myocardial infarction is complex involving several factors including smoking, systolic hypertension, dyslipidemia, history of diabetes, and psychosocial factors. Therefore, for the future studies, we suggested that these factors are controlled to determine the better outcome.

Our study had several limitations. First, some pivotal factors in thrombosis pathway including platelet function and the function of VAMP-8 expression were not measured. Second, study bias due to small sample size could drive to false negative findings. Third, samples in our study were only recruited from Saiful Anwar General Hospital. Fourth, several factors that might govern the gene polymorphism were not assessed, including environment, race, and lifestyle. Fifth, until now, the precise SNP affecting the level of VAMP-8 in the circulation is not well understood. Therefore, it might be difficult to assess the role of VAMP-8 gene polymorphism on the risk of acute myocardial infarction. Further studies with larger and well-characterized population are required to determine real effect of VAMP 8 gene polymorphism on the risk of acute myocardial infarction.

Conclusions

Our data suggest that, in our population, VAMP-8 A/G gene polymorphism is not associated with the risk of acute myocardial infarction. Moreover, VAMP-8 A/G gene polymorphism also has no correlation with acute myocardial infarction patients with smoking habits, hypertensive subjects, and premature acute myocardial infarction. Our results may contribute the better understanding concerning the VAMP-8 A/G gene polymorphism in acute myocardial infarction. However, further studies are required to determine the better outcome with eliminating the limitation factors.

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Conflicts of interest

The authors declare there are no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in
accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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